

Article

The delta subunit of rod-specific photoreceptor cGMP phosphodiesterase (PDE6D) contributes to hepatocellular carcinoma progression

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Supplementary Material

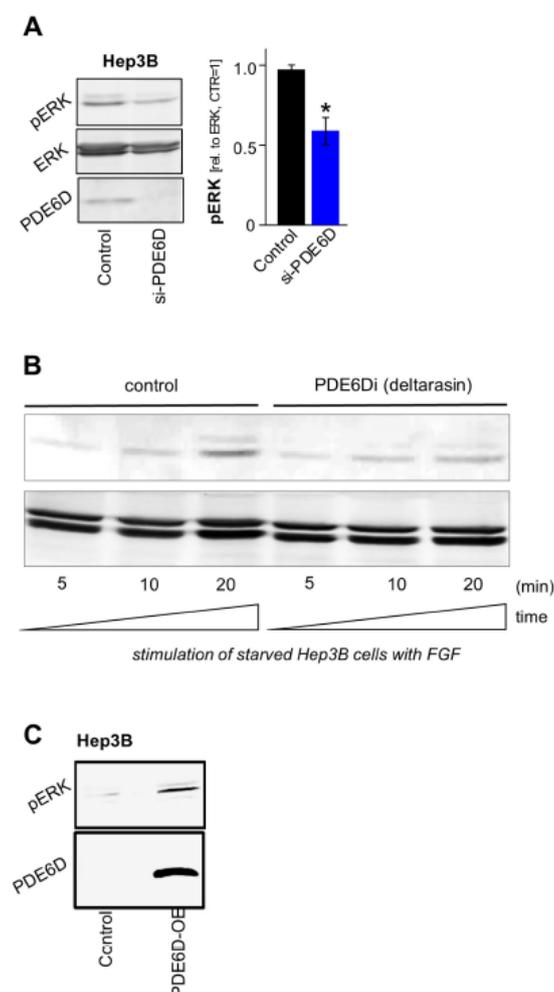


Figure 1. PDE6D-mediated ERK-activation in HCC. (A) Exemplary image (right side) and densitometric analysis (left side) of ERK-activation (pERK/ERK-levels) (Western blot analysis) in Hep3B cells after si-RNA-mediated PDE6D-suppression (*: $p < 0.05$ vs control). (B) Western blot analysis (exemplary image representing two independent experiments) revealing time-dependent recombinant Fibroblast-growth-factor (FGF)-induced ERK-activation (pERK/ERK-levels) in starved HCC cells (Hep3B) (cells were starved for 24 hours prior to stimulation with FGF) was prevented by co-treatment with the pharmacologic inhibitor of the PDE6D-KRAS-interaction deltarasin ($5 \mu\text{M}$). (C) Phospho-ERK (pERK) and PDE6D levels after forced PDE6D overexpression in sorafenib-resistant HCC cells (Hep3B).

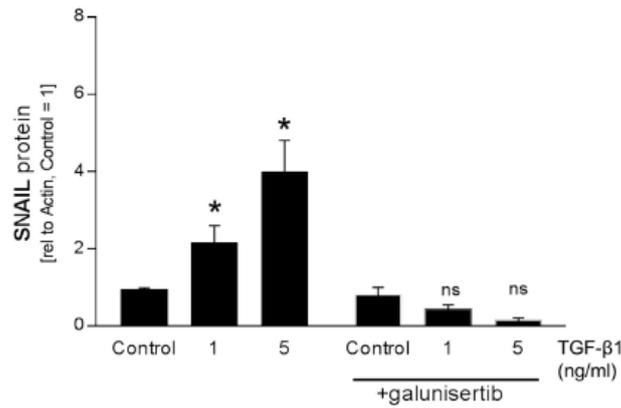


Figure S2. Snail protein expression after TGF-β1-mediated stimulation of HCC cells. Densitometric analysis of Snail protein expression levels (Western blot analysis) in PLC cells that were stimulated with different doses (0, 1, 5 ng/ml) of recombinant human TGF-β1 for 72 hours, with or without co-treatment with 15 μM of the TGF-β-receptor-1 (TGFBR1) inhibitor LY2157299 ("galunisertib") (the densitometric values represent two independent Western blot analysis) (*: $p < 0.05$ vs control; ns: non-significant vs control).

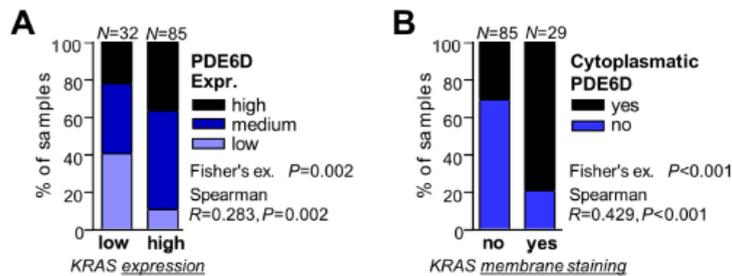


Figure S3. KRAS expression and membrane localization in human HCC tissues as correlated with PDE6D expression and cytoplasmic localization. (A) Tissue micro array analysis of PDE6D expression levels (high, medium, low) in human HCC tissues correlated with KRAS expression (high, low). (B) Tissue micro array analysis comparing KRAS membrane staining ("yes" vs "no") in human HCC tissues with ("yes") and without ("no") cytoplasmic localization pattern of PDE6D.

A

symbol	localization		HCC-related (PubMed)	Pathway/Function
	nuclear	non-nuclear		
ARL15				Signaling, Ras-family
ARL2			27798868	Signaling, Ras-family
ARL3				Signaling, Ras-family
CDC42			25978354	Cell cycle, mitosis
CETN3				Cell cycle, mitosis
COP55			27524414	Signaling
CUL1			26097587	Protein degradation
→ E2F1	■		28474358; 28134624	TF
FAM219A				nd
GNAI1			23691483	Signaling
GRK1				Rhodopsin-signaling
GRK7				Rhodopsin-signaling
→ HNF4A	■		29566023; 28498607	TF
HRAS			29423069	Signaling
KRAS			29275358	Signaling
→ MAX	■		29740493	TF
→ MYC	■		29893492	TF
PDE6A				Rhodopsin-signaling
PDE6B				Rhodopsin-signaling
PTGIR				nd
RAB13				Signaling, Ras-family
RAB18			23471881	Signaling, Ras-family
RAB8A				Signaling, Ras-family
RAD23A				Signaling, Ras-family
RAP1A			27780730	Signaling, Ras-family
RAP2B			28081729	Signaling, Ras-family
RASA1			26126858	Signaling, Ras-family
RHEB			29467900	Signaling, Ras-family
RHOA			29954442	Signaling, Ras-family
RHOB			28042950	Signaling, Ras-family
RND1				Signaling, Ras-family
RPGR				Signaling, Ras-family
→ TAF1	■			TF-associated
UBC				Protein degradation

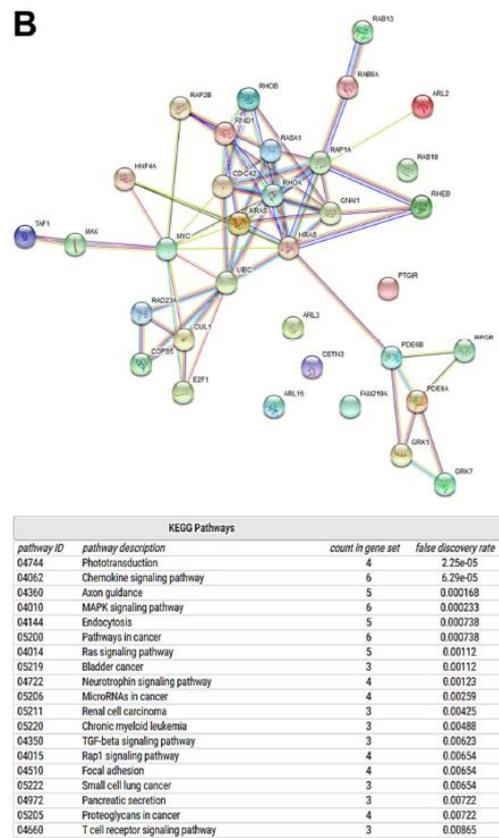
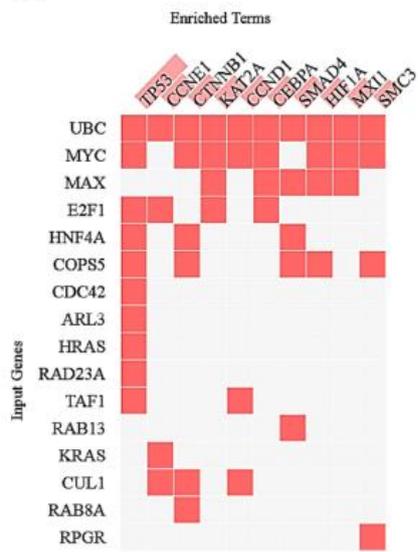
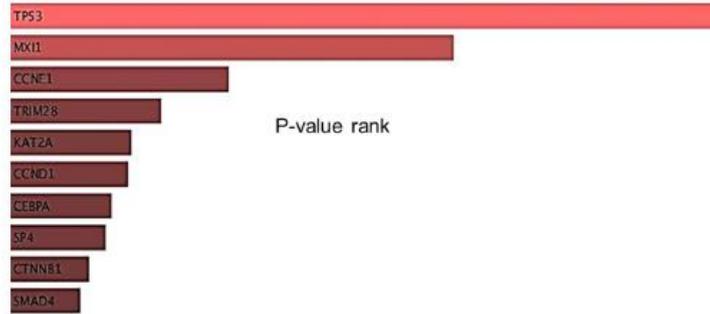


Figure 4. Potential protein-interactions of PDE6D and PDE6D-interactome dependent pathways. (A) Analysis of protein-protein interaction datasets was performed using the "Harmonizome" database. The table depicts potential PDE6D-interacting proteins that were derived from low-throughput or high-throughput studies from the following databases: Reactome, NCI Pathways, PhosphoSite, HumanCyc, HPRD, PANTHER, DIP, BioGRID, IntAct, BIND, Transfac, MiRTarBase, Drugbank, Recon X, Comparative Toxicogenomics Database, and KEGG. The table also depicts information about cellular localization, HCC-relation according to literature (Pubmed-IDs) and related pathways/functions. The black arrows mark nuclear proteins. (B) String database and DAVID bioinformatics database-derived graphical illustration (top panel) as well as Gene enrichment based Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (bottom panel) of the PDE6D-interactome.

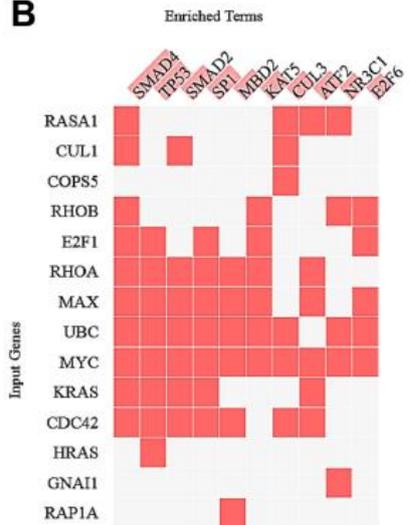
A



Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	TP53	5.758e-8	0.00001226	-1.92	32.05
2	CCNE1	0.00001108	0.0007889	-1.49	16.99
3	CTNNB1	0.00005018	0.001165	-1.88	16.67
4	KAT2A	0.00003173	0.001116	-1.54	15.96
5	CCND1	0.00003291	0.001116	-1.53	15.79
6	CEBPA	0.00003931	0.001116	-1.53	15.55
7	SMAD4	0.00005512	0.001165	-1.58	15.53
8	HIF1A	0.00008016	0.001165	-1.45	14.07
9	MXI1	9.772e-7	0.0001041	-1.01	13.92
10	SMC3	0.00008331	0.001479	-1.35	12.64



B



Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	SMAD4	4.584e-11	6.152e-8	-1.80	42.94
2	TP53	2.944e-8	0.00001975	-1.70	29.50
3	SMAD2	5.922e-7	0.0001987	-1.78	25.52
4	SP1	5.922e-7	0.0001987	-1.76	25.28
5	MBD2	0.00001004	0.001684	-1.85	21.26
6	KAT5	0.00001004	0.001684	-1.72	19.81
7	CUL3	0.00001004	0.001684	-1.66	19.06
8	ATF2	0.00001004	0.001684	-1.64	18.91
9	NR3C1	0.0001408	0.007269	-1.85	16.40
10	E2F6	0.0001408	0.007269	-1.83	16.20

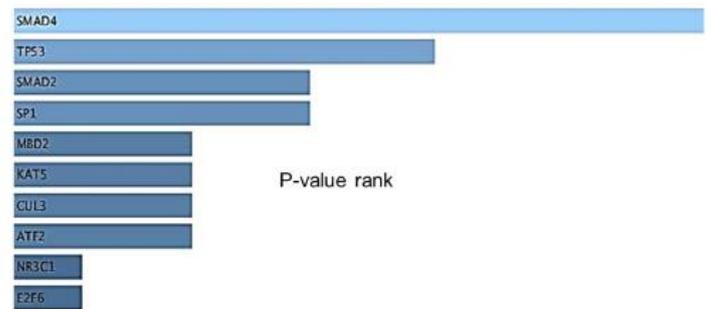


Figure S5. Potential transcription factor interactions of the PDE6D-interactome. Gene enrichment analysis using the "Enrichr" database depicting significantly enriched transcription factor terms for the 34 PDE6D-interacting protein list (Figure S1) in the "Enrichr Submissions TF-Gene Cocurrence" dataset (A) and the "Transcription Factor PPIs" dataset (B).